United States Environmental Protection Agency

LABORATORY ANIMAL PROJECT REVIEW

Please note:

1. All information in this LAPR is considered privileged and confidential by the IACUC and regulatory authorities.
2. Approved LAPRs are subject to release to the public under the Freedom of Information Act (FOIA). Do not include proprietary or classified information in the LAPR.
3. An approved LAPR is valid for three years.

LAPR Information

LAPR Title:	Pilot study to verify function and to develop operating and analytical procedures for running acoustic startle (AS) reflex test in rats.
LAPR Number:	18-06-003
Principal Investigator	Exemption 6
Author of this Document:	Exemption 6/RTP/USEPA/US
Date Originated:	05/14/2015
LAPR Expiration Date:	06/30/2018
Agenda Date:	07/15/2015
Date Approved:	07/27/2015
Date Closed:	05/01/2018

APPROVALS

APPROVER	NAME	APPROVAL DATE	COMMENTS	
	Exemption 6 Exemption 6 Exemption 6/RTP/USEPA/US by Exemption 6 /RTP/USEPA/US	07/27/2015	DMR	
	Exemption 6 Exemption 6 Exemption 6 RTP/USEPA/US by Exemption 6 /RTP/USEPA/US	07/27/2015	DMR	

Administrative Information

1. Project Title (no abbreviations, include species):
Pilot study to verify function and to develop operating and analytical procedures for running acoustic startle (AS) reflex test in rats.

Is this a continuing study with a previously approved LAPR?
No

2. Programatic Information

a. What Program does this LAPR support? Please provide the Research Program, Project, Task Number and Title.
Sustainable and Healthy Communities (SHC); Project Title: Assessing Environmental Health Disparities in Vulnerable Groups; Task Title: Early life environments: Impacts on life-long health; Task 2.6.3. ·Product Title: Interactions of chemical and nonchemical environmental stressors that impact children's healthy development and well-being

b. What is the Quality Assurance Project Plan (QAPP) covering this project?
In development DRAFT Title: Method development and validation of new test methods to assess cognitive functions, behavior, and affect in rodents.

3. EPA Principal Investigator/Responsible Employee:

Principal Investigator	***Phone Number***	***Division***	***Mail Drop***
Exemption 6	Exemption 6	TAD	MD
	Lotus Notes Address	***Branch***	B105-04
	Exemption 6 Exemption 6 Exemption 6 Exemption 6 Exemption 6 RTP/USEPA/US	NB	

4. Alternate Contact:

Alternate Contact	***Phone Number***	***Division***	***Mail Drop***
Exemption 6	Exemption 6	TAD	MD
	Lotus Notes Address	***Branch***	B105-04
	Exemption 6 Exemption 6 Exemption 6 Exemption 6 RTP/USEPA/US	NB	

SECTION A - Description of Project

1. Explain the study objective(s) in <u>non-technical language</u> such that it is understandable by non-scientific persons. <u>Explain how the benefits from the knowledge gained from this research outweigh the costs to the animals used in this research.</u> If this is a continuing study from a previous LAPR, briefly justify the continuation. Please spell out all acronyms and abbreviations with their initial use.

Background: The SHC task 2.63 focuses on characterizing the negative and positive impacts of early life environments on subsequent development and health. The EPA must consider children's unique sensitivities and vulnerabilities to prevent or reduce health disparities in its regulatory decisions and environmental programs. Children are often more sensitive to environmental chemicals, and non-chemical factors may exacerbate or reduce a chemical's adverse effects. Additionally, early life events such as nutritional imbalance or chemical exposure can alter physiological programming, resulting in increased disease risk later in life. This SHC task will develop animal models to discover and characterize health effects resulting from exposures of pregnant dams and offspring to combined chemical and non-chemical stressors.

Animal models are needed to enable elucidation of interactions among stressors in modifying adverse effects of developmental exposures. Such models allow long-term monitoring for latent effects impacting life-long health. In humans and animals, studies have shown that increased maternal emotional stress during pregnancy activates the Hypothalamic-Pituitary-Adrenal (HPA) axis and the Sympatho-Adrenal Medullary axis. Activation of the HPA Axis during gestation can impact fetal brain development, resulting in dysregulation of the HPA axis (response to stress), as well as increased emotionality and impaired cognitive functions.

Recently, we have acquired the acoustic startle reflex (ASR) equipment and relocated it to room A356. The equipment had to be completely disassembled and re-assembled. The ASR test, is used to test the startle reflex (SR), a contraction of the muscles in response to a sensory stimulus (loud, brief noise), and pre-pulse inhibition (PPI), modulation of the SR that occurs when a weak stimulus or "pre-pulse" occurs 30-500 msec prior to the startling stimulus. SR may be modulated by the affective state of the animal (i.e. increased anxiety or fear) and thus has been used in maternal stress research. PPI is reflective of a decrement in sensorimotor gating, that is, the ability to suppress sensory, cognitive or motor processes. Deficiencies in sensorimotor gating are observed with certain psychiatric and neurological disorders (i.e. schizophrenia, Huntington's Disease, OCD). The SR can be measured automatically in humans and rats using similar stimulus parameters. Thus, this behavior can be reliably quantified and compared across species.

After testing the equipment for basic functionality using calibration equipment, we wish to verify similar biological responses in adult animals with previous laboratory data and the literature. We would like to test the sensitivity of these measures with a positive control anxiogenic stimulus (light applied during testing). Unanticipated bright light is a arousing stimulus for rats. By varying the light intensity during ASR testing, the SR amplitude should change. This validation method is reported in Davis et al., 1996 to validate the sensitivities of the acoustic startle amplitude to anxiety.

Both the PI (see attached) and primary Research Support Scientist (RSS; **Exemption 6**) have experience using the AS procedure. Eventually the ASR will be incorporated into SHC 2.63 research plans. Training of other personnel on this equipment will commence after these protocols have been established.

2. Scientific rationale for proposed animal use.

a. Why is the use of animals necessary?

We will test the function of the AS equipment using calibration procedures. However, biological responses such as PPI and habituation cannot be quantified with calibration equipment.

b. Justify the species requested:

Rats have been used predominantly for maternal/prenatal stress research due to similarities to the human central and peripheral nervous systems and the similarities in their responses to different stressors including neurotoxicants. Rats over mice are preferred for cognitive testing due to ease of training on these complex tasks.

3. How was it determined that this study is not unnecessary duplication?

This is a pilot study to verify proper function of the equipment and recommended operating and analysis procedures. This function is not duplicative.

SECTION B - In Vivo Procedures

1. Briefly describe the experimental design. Include descriptions of the age, weight and sex of the animals. Supplementary information may be attached at the end of the LAPR, but please include critical information within the body of the LAPR.

Male and female, Long Evans rats, age 60 days will be used for pilot studies. Animals will be pair housed by sex, upon arrival, and allowed to acclimate for at least 7 days prior to beginning on the ASR tests.

Acoustic Startle Reflex (ASR): To test the startle response, rats are placed inside the test apparatus and a brief white noise burst (S2) produces the startle reflex. The startle reflex magnitude is recorded.

Pre-Pulse Inhibition (PPI): In this experimental paradigm, a non-startling stimulus or "prepulse" (S1; i.e., auditory, visual or tactile) is presented before the startle stimulus (S2), producing a decrease in the amplitude of the ASR. The degree of ASR reduction is related to the animal's ability to detect the S1. By varying the intensity of the S1, a threshold for detection can be established.

Psychological Stressor: In this experimental paradigm, the animals will be tested in the presence and absence of a light, to induce mild psychological stress and an increase in the ASR.

A daily test session lasts about a maximum of 75 min.

2. Justify the number of animals. Include explanation (e.g., biological, statistical, regulatory rationale) for the number of animals needed for each treatment group, and the overall number requested for the duration of the LAPR.

20 rats (n=10/sex) are needed to supply a large enough number to see differences with the given manipulations. This will be verified during the pilot studies, and power calculations can be performed to adjust the sample sizes in future SHC studies.

3. State how many animals over the study period are expected to be used under the following three categories of pain/distress (USDA nomenclature as defined in the instructions): Please enter numbers only.

Categories	Adults	Offspring
C) Minimal, transient, or no pain/distress:	20	
D) Potential pain/distress relieved by appropriate measures:		
E) Unrelieved pain/distress:		

4. Does this LAPR include any of the following:

- ☐ Restraint (>15 Minutes)
- ☐ Food and/or water restriction (>6 Hours)
- ☐ Survival surgery
- ☐ Non-survival surgery

5. Category C procedures. Describe each procedure separately, include details on the following:

a. Treatments (e.g., dosages, duration of exposure, route, volume, frequency):

No drugs or environmental agents are administered in these pilot studies.

b. Survival Blood Collections (method, volume, frequency):

None

c. Testing methods (including non-stressful dietary restrictions/modifications, mild non-damaging electric shock):

Acoustic Startle Reflex (ASR): To test the startle response, rats are placed inside a small aluminum cage (225L X 80W X 85H mm) mounted on top of a transducer inside a larger sound attenuated chamber. Animals are allowed to acclimate in the chamber for about 10 min prior to the start of the test session with a low background noise level 30 dBA - near ambient levels). A brief noise (S2; 120 dBA, about 40 msec duration) produces the ASR. The downward force on the sensor is recorded as the startle response. A total of 50 trials are given, each separated by a 20 sec inter-trial interval. This allows us to follow the startle response of the rat as it acclimates to the eliciting stimulus over time. Differences in the startle response can indicate changes in neuromuscular function

and can reflect increased or decreased reactivity to chemicals or stress (HPA axis regulation). One test session will be performed to assess startle response, gather data for analysis and create operating procedures (OPs).

Pre-Pulse Inhibition (PPI): A non-startling stimulus or "prepulse" (S1) is presented about 90 mses before the startle stimulus (S2). The S2 will be a 16 kHz tone presented using 23 intensities of S1 (-6 to 90 dB SPL in 3-6 dB increments) in the presence of ambient noise levels. Ten trials at each of the S1 intensities, and 10 trials in the presence of S2 alone will be recorded. Trials will be presented in a balanced, random format - so that in each of the ten 24 trial blocks each S1-S2 combination will only be presented once. One PPI session will be performed to verify function of pre-pulse equipment, develop protocols, OPs, and analysis protocols.

Psychological Stressor: In this experimental paradigm, the animals will be tested in the presence and absence of a light (LED lights approximating a 150W bulb), to induce mild psychological stress and an increase in the ASR. Two ASR sessions will be performed on 2 consecutive days. Rats will be tested under 2 conditions/day. On the first day, half of the rats will be tested in the dark, followed by testing in the light. The other half of the animals will be tested using the reverse order (light 1st, dark 2nd). On the second day the two conditions will be reversed for the respective groups, to control for habituation and/or run order effects. Each animal will undergo two sessions (days) of testing.

d. Animal restraint and confinement beyond routine housing and handling. Include a description of the type of restraint device, acclimation to device, duration of restraint:

e. Breeding for experimental purposes (e.g. length of pairing, number of generations):
NA

f. Describe how animals will be identified and monitored. Include description of identification procedures. (For example, if transponders are used, how are the animals prepared?) Include frequency of observations and by whom:
Rats will be ear tagged by animal care staff upon arrival from the breeder. Each animal will be identified and weighed twice a week by the animal care staff. Weights will be monitored for weight loss using animal weigh sheets and animals will be observed twice a week for any signs of distress or morbidity. Animals will be handled daily for 3-5 days prior to beginning AS tests to acclimate the animal to the researcher, On testing days animal weights and IDs will be confirmed prior to placement inside the AS chamber.

6. Non-surgical Category D or E procedures. Describe each procedure separately, include details on the following (Also fill in Section B.9).

a. Treatments (e.g. dosages, duration of exposure, route, volume, frequency):

b. Blood Collection (Provide a description of the procedure including method, volume, and frequency if appropriate. Indicate if the procedure is survival or terminal. Include preparatory methods, descriptions of incisions, etc.):

c. Testing methods:

d. Restrictions placed on the animals' basic needs (e.g., food and/or water restriction, light cycles, temperature). Provide details regarding the length of restriction. Describe the method(s) for assessing the health and well-being of the animals during restriction. (Amount of food or fluid earned during testing and amount freely given must be recorded and assessed to assure proper nutrition.):

e. Describe how animals will be monitored (e.g., frequency of observations, by whom):

f. Analgesia (Category D Procedures) - list drugs, dosages, route of administration and frequency:

g. If treatment-related deaths are expected, this must be thoroughly justified. Death as an endpoint is highly discouraged:

7. Surgical Category D and E procedures. Indicate if the surgery is survival or terminal. Describe each

surgical procedure separately, include details on the following (Also fill in Section B.9)

a. Complete description of surgical procedure including presurgical preparation, aseptic technique, surgical closure, etc:

b. Anesthetic regimen (Drugs, dosages, volume, route of administration and delivery schedule). The use of paralytic or neuromuscular blocking agents w/o anesthesia is prohibited:

c. Postoperative care (thermal support, special feeding, responsible personnel, removal of sutures/staples, frequency and duration of monitoring including weekend and holiday care):

d. Post operative analgesics (drugs, dosage, and volume and route of administration, frequency):

e. Will any animal be subject to more than one surgical procedure over the course of its lifetime, either here at NHEERL or elsewhere?

○ Yes ● No

f. Identify any surgical procedures performed at other institutions or by vendors:

8. Humane interventions (for treatments/procedures in all categories).

a. What resultant effects, if any, do the investigators expect to see following procedures or treatment? Please include transitory as well as permanent effects. Examples might include lethargy, ataxia, salivation or tremors. Indicate the expected duration of these effects.

No adverse effects are expected from procedures conducted in these animals.

b. State the criteria for determining temporary or permanent removal of animals from the study. Describe actions to be taken in the event of deleterious effects from procedures or chemical exposures. Describe actions to be taken in the event of clinical health problems not caused by procedures or exposures.

No procedures listed on this LAPR are expected to cause deleterious effects. If however, we find that an animal is losing weight (down more than 20 g from previous day, for 2 days), or shows sign of ill health (poor grooming, lethargy, etc..) we will consult with the veterinarian for our options of treatment. No animal will be tested under conditions of ill health, but can be included at a later time if it regains healthy status.

9. Alternatives to pain and distress (Category D and E Procedures only). Provide narrative regarding the sources consulted to ascertain whether acceptable alternatives exist for potentially painful/distressful procedures. Include databases searched or other sources consulted, the date of the search and years covered by the search, and key words and/or search strategy used. Assistance with searches is available through the EPA Library Staff.

SECTION C - Animal requirements

Describe the following animal requirements :

1. Indicate the number of animals required over the study period for this protocol. Please enter numbers only.

a. Animals to be purchased from a Vendor for this study: 20

b. Animals to be transferred from another LAPR:

LAPR Number that is the source of this transfer:

c. Animals to be transferred from another source:

d. Offspring produced onsite (used for data collection and/or weaned):

e. TOTAL NUMBER of animals for duration of the LAPR 20

2. Species (limited to one per LAPR): Rat(s)

3. Strain: Long Evans Rat(s)

Describe special requirements for animals with altered physiological responses (e.g., genetically altered, aged)

NA

4. Sources of animals:

Charles River, Raleigh

5. Provide room numbers where various procedures will be performed on animals:

Exemption 6
Exemption 6
Exemption 6

6. Will any animals be housed in areas other than the animal facility longer than 12 hours? If so, state location. Such areas require prior IACUC approval as a satellite facility before LAPR can be reviewed.

No *Room Numbers:*

7. Describe any transportation and containment methods involved in moving animals between EPA buildings, or between EPA and other institutions (excluding any commercial shipments)

NA

8. Describe any unusual housing or husbandry requirements, or acclimation requirements. Justify any treatment beginning less than 3 days after arrival.

NA

9. Describe special assistance requested of the animal contract staff, including procedures and dosing. NOTE, this request must be submitted separately to the Animal Resources Program Office (ARPO)

Animal care staff will be asked to weigh animals on a biweekly basis so that weights can be monitored.

10. Housing and Enrichment.

The IACUC encourages the use of environmental enrichment whenever possible (see IACUC website for details). Provide details on how the animals will be housed, including type of cage (e.g., solid bottom or wire screen), bedding material, number of animals per cage, and environmental enrichment. Note that housing rodents individually without environmental enrichment requires justification.

Animals will be housed 2 per cage by sex, using pine shaving bedding, and provided with Enviro Dry for enrichment.

SECTION D - Agents Administered to Animals

1. Identify all hazardous and non-hazardous agents to be administered to living animals. For agents requiring a Health and Safety Research Protocol (HSRP), provide the title of the approved HSRP for each such agent. If no protocol is required for an agent deemed potentially hazardous (e.g. nanoparticles, recombinant DNA), describe the safety precautions to be used.
Provide maximum dosing levels and route-appropriate LD50s (where available) for each agent used for dosing.

NA

2. Describe compounds to be administered to animals.

a. Are all substances pharmaceutical grade? If not, provide a scientific justification for the use of non pharmaceutical grade compounds.

NA

b. Describe any plans to administer human or animal tissues, blood or body fluids to the animals in the LAPR. Provide information to assure that such material is pathogen free. Indicate what safety precautions are necessary for handling the material.

NA

c. Provide a statement regarding any safety precautions necessary for handling any of these materials.

NA

NOTE: Any unresolved health/safety questions which arise during IACUC review of this LAPR will require consultation with the Safety, Health, and Environmental Management Office.

SECTION E - Personnel Training and Experience

1. Identify all project personnel conducting animal experimentation. Specify the techniques for which they have responsibility, and their relevant training and experience. Additional personnel may be added to the table below as a group (by Division) for Category C procedures. By so doing you are giving assurance that these personnel have received all required training and are qualified to perform the Category C techniques requested.

Use this area to type in additional personnel information not available in the table drop-down lists:

Hint: *The names in the first 2 lines of the table below are filled automatically from the Principal Investigator & Alternate Contact fields. A new line will be made available when a name is selected & upon leaving the name field (i.e. tabbing or clicking in another field).*

NAME	ROLE	SPECIFIC RESPONSIBILITY	RELEVANT TRAINING
Exemption 6	Principal Investigator	Oversight	About 30 years toxicology testing experience. EPA IACUC trained. Experience with surgical, behavioral, and neurophysiological testing.
Exemption 6	Technical Staff	Acoustic Startle testing Blood collection	Twenty-two years experience in neurobehavioral testing procedures in rodents. Some previous experience with Acoustic Startle test. EPA IACUC trained. Experience includes surgical procedures, intracranial infusions, sc and ip dosing, tail nick and vein blood draws..
Exemption 6	Technical Staff	Assist with blood collection	About 19 years neurotoxicological testing experience. EPA IACUC trained. Experience with physiological and behavioral techniques and surgical procedures, blood draws using tail nick and venipuncture.
Exemption 6	Technical Staff	Acoustic Startle testing	About 7 years neurotoxicological testing experience. EPA IACUC trained.
RTP-NHEERL	Tech Support	Category C Procedures	All NHEERL required training is complete.

SECTION F - Animal Breeding Colonies

This section pertains to the breeding of animals for maintenance of ongoing animal colonies. Do not include breeding that is part of experimentation and accountable under Section C.

Describe:

1. Estimated number of breeding pairs and liveborn per year

2. Breeding protocols and recordkeeping

3. Methods for monitoring genetic stability

4. Disposition of all offspring and retired breeders that are not used in accordance with the procedures described in this LAPR

SECTION G - Euthanasia

1. When will the animals be euthanized relative to experimental procedures?

Animals will be euthanized (CO2) when all pilot studies have been completed, but no later than when the rats are 1 year old.

2. Describe the euthanasia techniques:

Method(s): Euthanasia plus exsanguination
Agent(s): CO2
Dose (mg/kg):
Volume:
Route:

Source(s) of information used to select the above agents/methods:

Veterinary Staff

3. Provide justification and references for any euthanasia agent or method that is not consistent with recommendations of the American Veterinary Medical Association (AVMA) Guidelines for Euthanasia (e.g., cervical dislocation or decapitation without anesthesia; cervical dislocation in rodents weighing more than 200 grams).

4. Describe how death is to be confirmed.

Prolonged absence of breathing

SECTION H - Disposition of Used and Unused Animals

Describe the disposition of any animals remaining after project completion.

Transferred to another study if possible otherwise euthanized.

The IACUC encourages investigators to reduce the overall number of animals used at NHEERL. Would you consider transferring any unused animals from this LAPR to another approved LAPR?

● Yes ○ No

SECTION I - Assurances

1. Animals will not be used in any manner beyond that described in this application without first obtaining formal approval of the IACUC.

2. All individuals involved in this project have access to this application, are aware of all EPA policies on animal care and use, and are appropriately trained and qualified to perform the techniques described.

3. Thorough consideration of the three "R"'s (Replacement, Reduction, Refinement) has been given,

as applicable, to a. the use of animals, and b. procedures causing pain or distress (with or without analgesia/anesthesia), including death as an endpoint. The minimum number of animals required to obtain valid experimental results will be used.
4. The Attending Veterinarian has been consulted in regard to any planned experimentation involving pain or distress to animals.
5. The IACUC and Attending Veterinarian will be promptly notified of any unexpected study results that impact the animals' well-being, including morbidity, mortality and any occurrences of clinical symptoms which may cause pain or indicate distress.
6. All procedures involving hazardous agents will be conducted in accordance with practices approved by the Safety, Health, and Environmental Management Office.
7. I certify that I am familiar with and will comply with all pertinent institutional, state and federal rules and policies.
8. The IACUC has oversight responsibilities for animal care and use, and may request consultation or feedback regarding the conduct of in vivo procedures, progress and accomplishments, and any problems encountered.

EPA Principal Investigator	Certification Signature Date
Exemption 6 Exemption 6	07/21/2015

Submitted: 06/23/2015

Certification:

Certification by EPA Supervisor (Branch Chief or Division Director) that the project described herein has been reviewed and approved on the basis of scientific merit:

Branch Chief/Division Director	***Approval Date***	***Phone Number***	***Division***	***Mail Drop***
Exemption 6	06/24/2015 by Exemption Exemption Exemption Exemption 6 Exemption 6/RTP/USEPA/US	Exemption 6 ***Lotus Notes Address*** Exemption Exemption Exemption Exemption 6 Exemption 6/RTP/USEPA/US	TAD ***Branch*** DTB	MD ***Submitted to Branch Chief for Approval*** 06/23/2015 07:48 PM

ATTACHMENTS

LAPR 18-06-003 PI Resp Final.pdf

Exemption Exemption et al., 2006 Roles of the amygdala and bed nucleus of the stria ternminalis in fear and anxiety measures with the acoustic startle re

Exemptic Exemptic_COS_Toxsci_2007.pd Exemption 6 Exemption 6 etal_1987.pdf

Actions

First Update notification sent: 05/02/2016
Second Update notification sent:
First 2nd Annual notification sent:
05/02/2017
Second 2nd Annual notification sent:

1st Expiration notification sent: 04/26/2018
2nd Expiration notification sent:

History Log: